

NASA TECH BRIEF

Ames Research Center



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Enzymatic Regeneration of Adenosine Triphosphate Cofactor

The problem:

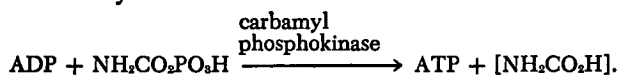
Recent advances in enzyme technology have provided a new class of catalysts which may be of great use in industrial processes. Current applications of the new technology are limited to simple enzyme processes, where the cost of the expended cofactor is of little concern; however, if more complex enzymatic reactions are to be given consideration as candidates for large-scale continuous processes, it is first necessary to develop techniques for regenerating the expensive cofactors in order to reduce catalyst costs to acceptably low values.

The solution:

Regenerate adenosine triphosphate (ATP) from adenosine diphosphate (ADP) by an enzymatic process which utilizes carbamyl phosphate as the phosphoryl donor.

How it's done:

The reaction between ADP and carbamyl phosphate to form ATP is catalyzed by carbamyl phosphokinase; the overall reaction may be represented essentially as:



A novel feature of the process is the extremely simple means of forming the carbamyl phosphate donor in the reaction medium.

The enzyme, carbamyl phosphokinase, is isolated from *Streptococcus faecalis* and partially purified. To permit its reuse, it is immobilized by attachment to porous glass: Porous glass, obtained as the alkylamine derivative, is treated for 30 minutes with a 2-percent aqueous solution of glutaraldehyde and then washed thoroughly to remove excess glutaraldehyde.

The derivatized glass is added to a solution of the enzyme, the mixture stirred gently for 2 hours, and then the enzyme-glass product is washed free of unreacted enzyme. The activity of the immobilized enzyme is 200 to 250 units per gram of glass (1 unit = 1 micromole of ATP formed per minute at room temperature).

The conversion of ADP to ATP takes place in a medium which contains potassium cyanate and potassium phosphate. Under these conditions, carbamyl phosphate forms spontaneously at room temperature and is utilized before decomposition can occur. Thus, when phosphate is used as buffer in the enzymatic reactions, only cyanate need be added along with ADP to regenerate ATP.

A small column containing 0.5 gram of the immobilized enzyme product was operated continuously for a period of 14 days. The solution pumped through the column was formed continuously from two stock solutions, one containing ADP, magnesium ions, and phosphate, and the other containing cyanate. The stock solutions were maintained at ice temperature and brought together in a mixing coil prior to entering the enzyme column. The column effluent was monitored periodically for ATP concentration by an enzymatic assay method. During the 14-day period, the activity of the column decreased by about 16 percent.

Note:

No additional documentation is available. Specific questions, however, may be directed to:

Technology Utilization Officer
Ames Research Center
Moffett Field, California 94035
Reference: B74-10057

(continued overleaf)

Patent status:

NASA has decided not to apply for a patent.

Source: David L. Marshall of
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